AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MINIREVIEW

Plague: Recognition, Treatment, and Prevention

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ABSTRACT Plague is caused by *Yersinia pestis* and is not commonly encountered in clinics, although natural plague foci are widely distributed around the world. *Y. pestis* has been listed as a category A bioterrorism agent. A neglected diagnosis will cause severe consequences. Therefore, this minireview briefly introduces the current understanding on *Y. pestis* and then focuses on practical aspects of plague, including clinical manifestations, diagnosis, treatment, and prevention, to alert clinicians about this notorious disease.

KEYWORDS plague, *Yersinia pestis*, clinical, diagnosis, treatment, prevention

Although plague is not common, it remains clinically important because natural plague foci are widely distributed throughout the world (1). Unfortunately, most modern clinical doctors neglect this notorious disease, although it is also a select agent with bioterrorism potential (2). Plague caused three major outbreaks in human history, changing the path of our civilization. The pathogen responsible for these three pandemics has been exclusively attributed to *Yersinia pestis*, according to high-throughput genome sequencing using ancient human remains (3, 4). In ancient times, plague spread was associated with human activities, such as maritime trading and the Silk Road, etc., which could transport fleas associated with live rodents and/or products that led to the plague spread (5, 6). Now, natural plague foci are widely distributed in Asia, Eurasia, Africa, and the greater American region. Since 2001, 14 major outbreaks have been reported to the World Health Organization (WHO) (http://www.who.int/csr/don/archive/disease/plague/en/), mainly from Africa and Asia. This review intends to provide practical information for clinical staff to recognize plague early and provide effective treatment and prevention (Fig. 1).

YERSINIA PESTIS, A PLAGUE PATHOGEN

Y. pestis, a Gram-negative bacterium, belongs to the family *Enterobacteriaceae*. It was first isolated during the third pandemic plague in Hong Kong by Alexandre Yersin. In addition to *Y. pestis*, the genus *Yersinia* includes *Y. pseudotuberculosis* and *Y. enterocolitica*, which are also associated with human infections and cause mild diarrhea (7). *Y. pestis* is a "young" pathogen that evolved from *Yersinia pseudotuberculosis* around 5,000 to 7,000 years ago by obtaining a flea-transmitted life cycle and the capability to cause host systemic infection (5, 6, 8); *Y. pestis* is now considered a clonally expanded genomically degenerating variant of *Y. pseudotuberculosis*. During its evolution, *Y. pestis* showed a varied mutation rate, not strictly obeying the constant evolutionary clock (6). Two newly acquired plasmids (pMT1 and pPCP1) and a *Y. pseudotuberculosis*-inherited plasmid (pCD1) play a critical role in *Y. pestis* pathogenicity, in addition to many chromosomal loci, such as *pgm*, which bears a high pathogenicity island with functions of iron acquisition and biofilm formation (9). The inherited plasmid pCD1 encodes a type III secretion system that is a needle-like structure on the bacterial surface and can inject toxic *Yersinia* outer proteins (Yops) into host cells upon contact between the pathogen and cells (10). Yops can inhibit the host's innate immunity and destroy host cell structures, playing key roles in plague pathogenesis. Specific to *Y. pestis*, however,

Accepted manuscript posted online 25 October 2017

Citation Yang R. 2018. Plague: recognition, treatment, and prevention. J Clin Microbiol 56:e01519-17. https://doi.org/10.1128/JCM.01519-17.

Editor Colleen Suzanne Kraft, Emory University

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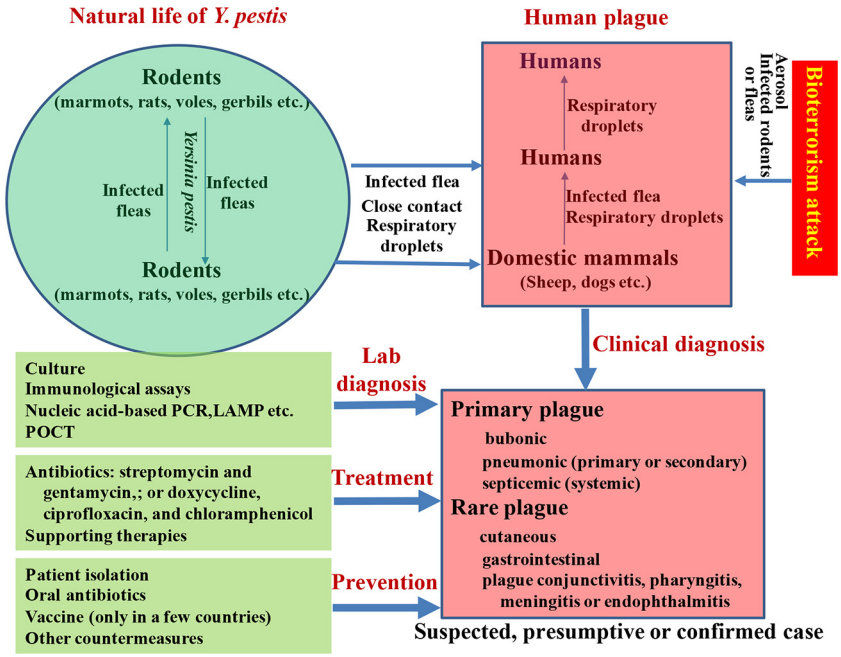

FIG 1 Transmission, diagnosis, treatment, and prevention of plague. Plague is a zoonotic disease caused by *Yersinia pestis*, whose natural life cycle includes rodents and fleas. Plague can be transmitted to humans through the bite of an infected flea, close contact with animals suffering from plague, or respiratory droplets of an infected animal. Patients with pneumonic plague can spread *Y. pestis* to close contacts by severe coughing. Bioterrorism attacks can also intentionally result in plague epidemics through aerosol release or infected animals/fleas. Clinically, plague primarily manifests as bubonic, pneumonic, or septicemic plague. Other rare forms of plague, such as cutaneous or gastrointestinal plague, have also been reported. According to the World Health Organization guideline, plague can be diagnosed as a suspected, presumptive, or confirmed case based on different kinds of evidence. Laboratory evidence includes detection by bacterial culture and antigen, antibody, and nucleic acid testing. Antibiotics, including streptomycin and gentamicin, are recommended to effectively treat the different types of plague. Preventive countermeasures include physical isolation of patients, oral antibiotics, and other strategies. LAMP, loop-mediated isothermal amplification; POCT, point-of-care testing.

the more recently acquired plasmid pMT1 encodes the well-known fraction 1 (F1) capsular antigen, which is used as a vaccine component and a diagnostic target, and the murine toxin (11), which is critical for bacterial survival in the flea gut. Another newly acquired plasmid, pPCP1, encodes a proteinase, plasminogen activator (Pla), which is critical for bacterial invasion into tissues (1).

HOW TO RECOGNIZE PLAGUE

Three major forms of plague are usually described, including bubonic, pneumonic, and septicemic plague (7). However, *Y. pestis* can be transmitted not only by flea bites (causing bubonic plague) and respiratory droplets (causing pneumonic plague), but also by the consumption of uncooked contaminated meat (causing gastrointestinal plague) and contact with infected pets/domestic animals (causing conjunctivitis, skin plague, or pneumonic plague) (12). In addition, plague pharyngitis, meningitis, and endophthalmitis have been reported, albeit rarely (13). If bubonic plague is not recognized and treated in time, it can develop into pneumonic plague or systemic plague (septicemic plague) by spreading *Y. pestis* via blood; this plague type has a very high mortality rate (7). Septicemic plague can also be caused directly by blood infection of the pathogen through a cut.

The first symptoms of plague are similar to those of the flu, with high fever (up to 39 to 40°C), malaise, chills, and headache. An important clue for suspecting plague is contact history with wild animals in natural plague foci or with other plague patients. If a patient develops sudden high fever after close contact with dead animals (rodents or other wild animals) in a region where plague is endemic (14), bubonic plague (with

regional lymph node swelling), pneumonic plague (with severe coughing and pneumonic signs by X ray), or septicemic plague (with sudden high fever and chills) should be highly suspected. The incubation period is generally 2 to 3 days but may be as long as 6 days (15). If the patient is infected by inhaling a large quantity of *Y. pestis*, causing primary pneumonic plague, the incubation period might be 1 day or less, and the symptoms and signs may progress very quickly (15). In cases of bubonic plague, patients usually develop regional red, dry, and hot skin, with progressive severe pain in the flea-biting region and forced position caused by severe pain of the swollen lymph nodes (16).

HOW TO DIAGNOSE PLAGUE

Clinical diagnosis. According to the guideline issued by the WHO, plague should be defined as a suspected, presumptive, or confirmed case (17).

For a suspected case, compatible clinical symptoms and signs should always be accompanied by typical epidemiological features, including a trip to an area endemic for the disease within 10 days before the onset of symptoms and signs or residence in such an area, exposure to plague patients or infected animals, and/or obvious history of flea bites. Although these risk factors are sometimes evident, clinical doctors or veterinarians likely misdiagnose plague because of a lack of experience in diagnosing *Yersinia pestis* infection in humans or animals (12).

For a presumptive case, the above-described clinical and epidemiological manifestations should be observed. Additionally, the patient exhibits the following criteria: Giemsa or Wayson staining of Gram-negative coccobacilli in samples from bubo aspirate, blood, or sputum, with a bipolar appearance suggestive of a safety pin; detection of F1 antigen from sputum, bubo aspirate, or blood; detection of serum anti-F1 antibody without history of previous plague infection or immunization; and PCR detection of *Y. pestis* in sputum, bubo aspirate, or blood. If the patient is not from a focus in which plague is endemic, he or she should be considered infected in a new or reemerging plague focus by having two positive results in the above-described laboratory examinations. If the patient is from a focus in which plague is endemic, one positive result is sufficient to diagnose the patient as a presumptive case.

For a confirmed case, in addition to meeting the criteria of a suspected case, the patient should meet the following criteria: *Y. pestis* isolated from bubo aspirate, blood, or sputum; *Y. pestis* identified by morphological, biochemical, phage lysis, F1 antigen detection, and PCR tests; and 4-fold increase in anti-F1 antibody titer in paired serum samples. Additionally, rapid tests, including an immunochromatographic strip test, may be used to confirm the existence of F1 antigen from clinical samples in areas in which plague is endemic and where laboratory support is unavailable for the performance of other confirmatory tests.

Laboratory diagnosis. The gold standard for plague diagnosis in the laboratory is the isolation and identification of the plague pathogen from clinical specimens (https://www.cdc.gov/plague/healthcare/clinicians.html). The pathogen can be cultivated on many routinely used media, including brain heart infusion broth, MacConkey agar, and sheep blood agar. *Y. pestis* grows optimally at 26 to 28°C; however, incubation at 37°C is necessary for F1 antigen production. The colonies formed on the agar plate after a 48-h incubation are small (about 1 to 2 mm in diameter), with raised centers and a flat periphery. *Y. pestis* appears as small pleomorphic rods by Gram staining and bipolar coccobacilli by Giemsa or Wayson staining. The cultures could be specifically lysed by *Y. pestis* phage at 22 to 25°C. *Y. pestis* is not active in terms of biochemical assays; therefore, conventional biochemical identification systems sometimes result in misidentification with *Y. pseudotuberculosis* or other enterobacteria (18). Isolation of *Y. pestis* should be performed at minimum in a biosafety level 3 laboratory.

F1 antigen is typically used as a target to detect *Y. pestis* by immunological methods. A passive hemagglutination test and F1 antigen hemagglutination inhibition test are conventionally employed for detecting F1 antigen (18). However, direct fluorescent

antibody testing and enzyme-linked immunosorbent assays have also been reported for detecting F1 antibody or F1 antigen quantitatively (19).

Y. pestis can be detected by PCR targeting the F1 antigen gene (*caf1*), *pla* gene, or chromosomal fragments (such as fragment 3a) (20, 21). However, the *pla* gene and chromosomal fragment targets were recently shown to be unreliable for detecting *Y. pestis* (22). Immunological and nucleic acid-based detection of *Y. pestis* can be performed in a biosafety level 2 laboratory.

For areas without a supporting laboratory to perform the above-described bacterial isolation, identification, and immunological or molecular detection assays, point-of-care testing will be helpful. Immunochromatographic assays (ICA) have been employed for rapid on-site detection (23). The colloidal gold-based ICA can meet the urgent need for on-site detection in remote centers; however, it must be conducted by well-trained professionals to ensure the accuracy of the results (23). An up-converting phosphor technology-based ICA has been developed with advantages of reliability, quantification, and robustness (24). Portable real-time quantitative PCR thermocyclers, such as PikoReal (Thermo Fisher Scientific, Inc., USA), may also be applied to detect plague pathogen in the field (25).

HOW TO TREAT PLAGUE

The keys to the successful treatment of plague are early recognition and timely administration of effective antibiotics. If the administration of effective antibiotics and antishock therapies are delayed by more than 24 h, it will usually be fatal for the patients. Most *Y. pestis* isolates worldwide are sensitive to streptomycin; however, a multidrug-resistant (MDR) strain was isolated from Madagascar (26). Fortunately, this MDR strain has never emerged again naturally in this region and other parts of the world. There is a concern that this MDR strain (resistant to streptomycin, chloramphenicol, ampicillin, spectinomycin, kanamycin, tetracycline, sulfonamides, and minocycline) may be attractive to bioterrorists who may want to perpetuate a bioterrorism attack using this strain. If infection by such a strain does occur, caution must be used in the selection of effective antibiotics by avoiding administration of the above-mentioned ones. The majority of human cases can be treated successfully with effective antibiotics according to the United States Centers for Disease Control and Prevention (CDC)'s recommendation.

Streptomycin and gentamicin are recommended for adult patients, including immunocompromised patients and pregnant women. Streptomycin and gentamicin may also be administered in children; however, the dosage should be reduced. Alternatively, the combination of doxycycline, ciprofloxacin, and chloramphenicol could also be used for both adults and children. For the detailed antibiotic administration protocol, please refer to the CDC training lessons (https://www.cdc.gov/plague/resources/Recommended-antibiotics-for-plague_revision-Aug-2015_Final-(00000002).pdf).

In a large-scale plague outbreak or bioterrorism attack setting, oral doxycycline and ciprofloxacin are recommended to treat the plague for both adult and child patients. Alternatively, chloramphenicol could also be chosen for treating adult patients; however, for children, the combination of chloramphenicol and ciprofloxacin should be used.

For the treatment of primary pneumonic plague, we have successful experience using the combination of streptomycin and ciprofloxacin (for detail, please refer to Table S1 in reference 15).

It is important to note that regulations on antibiotic administration vary by country. For instance, in Russia, ciprofloxacin is not allowed for children under 15 years old. Therefore, antibiotic treatment for plague may slightly vary by location as well.

For plague treatment, in addition to antibiotic administration, supportive therapy for severe symptoms, such as shock, should not be neglected (7). Other therapies have been reported, including immunotherapy, phage therapy, bacteriocin therapy, and

application of virulence factor inhibitors; however, they are not routinely used in clinics (7).

HOW TO PREVENT PLAGUE

Bubonic plague patients without secondary pneumonic and septicemic plague have a very low risk of spreading plague to close contacts. However, a patient with secondary or primary pneumonic plague can transmit *Y. pestis* to close contacts through coughing respiratory droplets. Within the first 24 h of the onset of pneumonic plague, the patient develops fever and quick heart rate without coughing and bloody sputum expectoration. This period is noninfectious (27). If a patient expectorates bloody sputum, it will be highly infectious. However, transmissibility by this route is not strong, because the estimated basic reproduction number, R_0, is on the order of 2.8 to 3.5 (28). It is relatively easy to prevent pneumonic plague, because wearing a face mask or even covering one's mouth with a jacket can effectively prevent transmission (15). However, a patient with suspected pneumonic plague or bubonic plague with secondary pneumonic or septicemic plague should be isolated (15).

Except for physical prevention, antibiotic prophylaxis using tetracycline, streptomycin, and chloramphenicol has been recommended by the WHO Expert Committee on Plague (1970) (29). There is still no effective vaccine for plague prevention, although a live attenuated vaccine is used in some countries, such as China and Russia (30).

As mentioned above, although plague is not commonly encountered in clinics, it will cause severe consequences if a timely correct diagnosis is neglected. Because *Y. pestis* is also an important bioterrorism agent and the perpetrators may intentionally use this pathogen to threaten societal stability, clinicians need to pay attention to this rare but harmful disease to prevent its further spread.

ACKNOWLEDGMENTS

Our research on *Yersinia pestis* is funded by the National Natural Science Foundation of China (grant 31430006).

We thank Christina Croney, from Liwen Bianji, Edanz Group, China (www.liwenbianji.cn/ac), for editing a draft of the manuscript.

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